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Plunkett
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Sornasse et al.

Title: **GENES REGULATED BY HUMAN CYTOKINES**

Serial No.: 09/435,247 Filing Date: 5 November 1999

Examiner: M. Sheinberg Group Art Unit: 1631

Commissioner for Patents
Washington, DC 20231

DECLARATION UNDER 37 CFR 1.132 OF SUSAN G. STUART

I, Susan G. Stuart, declare:

1. I received the Degree of Doctor of Philosophy in Genetics from the University of New Hampshire in 1983 and have had postdoctoral training in DNA replication at Albert Einstein College of Medicine (Bronx NY) and in molecular immunology at the DNAX Research Institute (Palo Alto CA). I have been employed at Incyte Genomics since May 1994. I was promoted from Director, managing the scientists and experiments which produced the LifeSEQ databases, into my current position as Senior Director of Research Technologies, where I established the scientific collaborations and contributed to the success of the Incyte expression database and technologies.

2. The application relates primarily to a composition comprising a plurality of polynucleotides, i.e. a combination, which were at least two fold up- or down-regulated in PBMC treated with cytokines.

3. I understand that the Examiner has rejected claims 1, 6-7, and 18-20 under 35 USC §§ 101 and 112 because the claimed invention lacks patentable utility.

4. The purpose of my declaration is to support the utility asserted in the specification that the expression of the cDNAs of the composition are diagnostic of "conditions, disorders and diseases associated with the immune system and immune response" as set forth on page 1, lines 6 and 7 of the specification. To that end, I provide the following transcript image for SEQ ID NO:219 as the elected, differentially expressed polynucleotide of the composition.

5. A "transcript image" (TI) is a profile of gene transcription activity in a particular tissue at a particular point in time. TI was used to assess the relative abundance of the expressed

polynucleotide among all ESTs in the cDNA libraries of LIFESEQ GOLD database as described in USPN 5,840,484.

In an attempt to explain the method, I provide the following information: 1) The criteria for transcript imaging can be selected from category, number of cDNAs per library, library description, disease indication, clinical relevance of sample, and so forth; 2) All sequences and cDNA libraries in the LIFESEQ GOLD database have been categorized by system, organ/tissue and cell type; and 3) For each library in a category, the number of cDNAs were counted and their abundance shown relative to the total number of cDNAs sequenced in that library or category.

The categories in which SEQ ID NO:219 was expressed are shown below. The first column shows category; the second column, the number of cDNAs sequenced in that category; the third column, the number of libraries in which the sequence was expressed over the total number of libraries; and the fourth column, abundance (or number of) transcripts in the library. The totals at the bottom of the table are for all instances of cDNAs and transcripts in the LIFESEQ GOLD database (Jan02 rel).

CATEGORY	cDNAs	FOUND IN	ABUND
Digestive	572415	1/164	1
Female Reprod	486361	1/123	1
Male Reprod	489837	1/129	1
Nervous	1051758	1/239	1
Mixed	200857	1/27	1
Totals	6054316	5/1538	5

In some transcript images, all normalized or subtracted libraries, which have high copy number sequences removed prior to processing, and all mixed or pooled tissues, which are considered non-specific in that they contain more than one tissue type or more than one subject's tissue, can be excluded from the analysis. Treated and untreated cell lines and/or fetal tissue data can be excluded where clinical relevance is emphasized. Conversely, fetal tissue may be emphasized wherever elucidation of inherited disorders or differentiation of particular adult or embryonic stem cells to form organs such as heart, kidney, nerves or pancreas would be furthered by removing clinical samples from the analysis.

The complete transcript image for SEQ ID NO:219 is shown below. The first column shows library name; the second column, the number of cDNAs sequenced in that library; the third column, the description of the library; the fourth column, abundance of the transcript in the library; and the fifth column, percentage abundance of the transcript in the library.

SEQ ID NO:219
Category: All

Library*	cDNAs	Description of Tissue	Abund	% Abund
COLNNON03	3335	colon, mw/adenoCA, aw/node mets, 60M, NORM	1	0.0300
OVARTUN01	3917	ovary tumor, mets colon adenoCA, 58F, NORM	1	0.0255
HIPONON02	6050	hippocampus, mw/intracranial hemorrhage, 72F, NORM	1	0.0165
PROSNOP05	21234	prostate, 32M, SUB, 3'CGAP	1	0.0047
MIXDTUP01	21225	mixed tissues, includes tumor, SUB, 3'CGAP	1	0.0047

*Since all libraries were normalized or subtracted which biased sequencing toward low copy number cDNAs, no libraries were removed from the analysis.

The descriptions of the tissues used in constructing the libraries in which SEQ ID NO:219 was differentially expressed are shown below.

COLNNON03 was constructed from 2.84 x 1e6 independent clones from the COLNNOT07 library. mRNA was made from colon tissue removed from a 60-year-old Caucasian male during a left hemicolectomy. Pathology indicated the margins of resection were free of involvement. Pathology for the matched tumor tissue indicated an invasive grade 2 adenocarcinoma, which extended through the submucosa superficially into the muscularis propria. One of 9 regional lymph nodes contained metastatic adenocarcinoma. Family history included atherosclerotic coronary artery disease in the mother and colon cancer in the sibling(s).

OVARTUN01 was constructed from 5.36 million independent clones from an ovarian tumor library. mRNA was made from tumor tissue removed from the left ovary of a 58-year-old Caucasian female during a total abdominal hysterectomy, removal of a single ovary, and inguinal hernia repair. Pathology indicated a metastatic grade 3 adenocarcinoma of colonic origin, forming a partially cystic and necrotic tumor mass in the left ovary, and forming a nodule in the left mesovarium. The endometrium was inactive. Patient history included colon cancer.

HIPONON02 was constructed from 1.13 million independent clones from a hippocampus tissue library. mRNA was made from the hippocampus tissue of a 72-year-old Caucasian female, who died from an intracranial hemorrhage. Patient history included nose cancer, hypertension, arthritis, and tobacco use. The patient was taking medication for hypertension.

By percent abundance, SEQ ID NO:219 was 4- to 7-fold more highly expressed in libraries made from tissues of patients with metastatic colon cancer or who died due to intracranial hemorrhage. These tissues are responding to cytokines produced in response to cellular invasion or tissue compression--conditions or diseases fully consonant with those listed for anti-inflammatory cytokines in the application. Furthermore, when used in a tissue-specific and clinically relevant manner with biopsied human tissues, SEQ ID NO:219 could be used as a diagnostic for metastatic colon cancer.

To further support the expression profiles and sequence categorization as revealed by microarray data, I would like to present one additional transcript image. The TI for SEQ ID NO:173, which belongs in the category of sequences showing expression induced by treatment with pro-inflammatory cytokines, is shown below.

SEQ ID NO:173
Category: Musculoskeletal

Library	cDNAs	Description of Tissue	Abund	% Abund
SYNORAB01	5051	synovium, hip, rheuA, 68F	3	0.0594
SYNORAT01	2412	synovium, elbow, rheuA, 51F	1	0.0415
SYNORAT04	5635	synovium, wrist, rheuA, 62F	2	0.0355
SYNORAT05	3465	synovium, knee, rheuA, 62F	1	0.0289
SYNORAT03	5786	synovium, wrist, rheuA, 56F	1	0.0173

When used in a tissue specific and clinically relevant manner, SEQ ID NO:173 is diagnostic of rheumatoid arthritis, a disease listed in the specification as associated with proinflammatory cytokines. SEQ ID NO:173 was not expressed in normal or osteoarthritic cartilage libraries (CARGDIT01, CARGDIT02, and CARGNOT01), in normal or osteoarthritic synovium (SYNONOC01, SYNONOT01, and SYNOOAT01), or in thirteen fetal, normal, or tumorous bone (BONENOP01, BONETUP01, BONETUP02, BONFTXN06, BONFTXT01, BONMTUE02, BONRFEC01, BONRFET01, BONRFET03, BONRNOT01, BONRTUT01, BONSTUT01, and BONTNOT01).

9. Although an example could be shown for each claimed sequence, I believe that the transcript images above clearly demonstrate the value of the expression profiles, pro- and anti-inflammatory disease associations, and the utilities asserted in the specification.

I hereby declare that all statements made herein are true and that they are based on my own knowledge, information and belief. These statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issued from it.

Date: 1 February 2002

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